Formulation and *In-vitro* Evaluation of Ciclopirox Ethosomal Gel

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**Abstract**

Ethosomal systems were lipid vesicular carriers which contain high percentage of ethanol and ethosomal gels were prepared in present study using ciclopirox as model drug. EF1-EF12 different formulations were prepared by varying concentration of ethanol from 20-50% and soya lecithin from 2-4%. Ethosome formulations were characterized for compatibility, drug entrapment efficiency, drug content, invitro drug release studies, surface morphology, vesicle size and size distribution. Ethosomal gel was prepared for optimized ethosomal formulation EF7 by incorporated into 1-2% carbopol gel. Ethosomal gels were characterized for pH, viscosity and spreadability. Optimized ethosomal formulation's in-vitro drug release pattern was studied for different release kinetic models.

**Keywords:** Ethosomal system, Lipid vesicular carriers, Surface morphology, Entrapment efficiency.

**Introduction**

Transdermal drug delivery systems are externally applied medicaments in the form of gels that deliver drugs for systemic effects at a predestined and controlled rate [1,2]. Through a diffusion process, the drug enters the bloodstream directly through the skin. Since there is high concentration in ethosomal gel and low concentration in the blood, the drug will keep diffusing into the blood for an extended period of time, maintaining the constant concentration of drug in the blood flow [3-7].

Transdermal delivery is an important delivery route that delivers precise amount of drug through the skin for systemic action [8]. Throughout the past two decades, the transdermal drug delivery has become a proven technology holding the promise that new compound could be delivered in a safe and convenient way through the skin.

**Ethosomes**

Vesicles would also allow to control the release rate of drug over an extended time, keeping the drug shielded from immune response or other removal systems and would be able to release just the right amount of drug and keep that concentration constant for longer periods of time (Figure 1). Ethosomal vesicles have more benefits in comparison to other transdermal delivery systems. Ciclopirox is an antifungal agent used in present study as model drug.

**Materials and Methods**

**Materials**

Drug ciclopirox was gifted by Chandra labs, Hyderabad. Soya lecithin and cholesterol were purchased from standard chemicals and reagents, Hyderabad. Carbopol, PG (propylene glycol), ethanol, TEA...
under stirring for 20-30min [12]. Ethosomes equivalent to 8% of Ciclopirox were collected and dispersed in the hydrated polymer slurry under stirring condition, continued for 20min (shown in tables 1,2). Adjust the pH of gel using phosphate buffer solution [13,14].

**Analytical study**

**Drug solubility:** Ciclopirox drug solubility was studied in different solvents. It is observed that drug ciclopirox is sparingly soluble in water, soluble in ethanol, methanol and phosphate buffer 6.8pH [15].

**Drug lambda max determination:** Ciclopirox drug scanned under UV visible spectrophotometer and it is observed that maximum absorbance at 212nm as shown in figure 2.

**Preparation of calibration curve for Ciclopirox:** Ciclopirox drug solutions were prepared in 2-10 µg/ml concentrations using 7.4pH phosphate buffer as solvent [16,17]. Absorbance noted for each concentration using UV Visible spectrophotometer at 212nm and plotted against concentration as shown in figure 3.

**Drug – Excipients compatibility studies (FTIR):** IR spectroscopy can be used to investigate and predict any physicochemical interactions between different components in a formulation and therefore it can be applied to the selection of suitable chemically compatible excipients [18-20]. Drug – Excipients compatibility studies were shown in figures 4 and 5.

**Evaluation Parameters**

**Evaluation of ethosomal vesicles**

**Vesicular characterization:** Ethosomes were visualized by using Scanning electron microscopy (SEM). Effect of lecithin and ethanol concentrations on the size and distribution of ethosome vesicles was investigated using complete size distribution analysis.

**Methodology**

**Preparation of ethosomal vesicles:** Ciclopirox ethosomal formulations were prepared (shown in table no.1) by ‘Cold method’, drug concentration was fixed to 8% w/v, vesicle forming agent soya lecithin concentration in formulation was varied from 2-4% and ethanol concentration was adjusted from 20-50%. In this method phospholipids and lipid materials were dissolved in ethanol at room temperature by magnetic stirring process with addition of penetration enhancer PG continuously. Add drug with constant stirring followed by heating the above mixture at 30°C in water bath. In separate vessel heat water at 30°C and mix both the phases together, stir for 5min. Vesicle size of ethosomes can be decreased by extending the sonication process [9-11]. Final formulation stored under refrigeration.

**Preparation of ethosomal gel:** Dissolve TEA in water under stirring and add carbopol slowly, allow hydrating (triethanolamine) and water were laboratory standards. Phosphate buffer 7.4pH was prepared as per pharmacopoeial guidelines.

<table>
<thead>
<tr>
<th>Ethosomal formulation</th>
<th>Lecithin (%)</th>
<th>Ethanol (%)</th>
<th>Propylene glycol (%)</th>
<th>Drug (g)</th>
<th>Cholesterol (g)</th>
<th>Water</th>
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</thead>
<tbody>
<tr>
<td>EF₁</td>
<td>2</td>
<td>20</td>
<td>10</td>
<td>0.080</td>
<td>0.005</td>
<td>q.s.</td>
</tr>
<tr>
<td>EF₂</td>
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<td>30</td>
<td>10</td>
<td>0.080</td>
<td>0.005</td>
<td>q.s.</td>
</tr>
<tr>
<td>EF₃</td>
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<td>40</td>
<td>10</td>
<td>0.080</td>
<td>0.005</td>
<td>q.s.</td>
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<td>EF₄</td>
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<td>50</td>
<td>10</td>
<td>0.080</td>
<td>0.005</td>
<td>q.s.</td>
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<tr>
<td>EF₅</td>
<td>3</td>
<td>20</td>
<td>10</td>
<td>0.080</td>
<td>0.005</td>
<td>q.s.</td>
</tr>
<tr>
<td>EF₆</td>
<td>3</td>
<td>30</td>
<td>10</td>
<td>0.080</td>
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<td>q.s.</td>
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<td>40</td>
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<td>0.080</td>
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<td>q.s.</td>
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<tr>
<td>EF₈</td>
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<td>0.005</td>
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<td>EF₁₀</td>
<td>4</td>
<td>30</td>
<td>10</td>
<td>0.080</td>
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<td>EF₁₁</td>
<td>4</td>
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<td>10</td>
<td>0.080</td>
<td>0.005</td>
<td>q.s.</td>
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<tr>
<td>EF₁₂</td>
<td>4</td>
<td>50</td>
<td>10</td>
<td>0.080</td>
<td>0.005</td>
<td>q.s.</td>
</tr>
<tr>
<td>*EF₁₃</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.080</td>
<td>0.005</td>
<td>q.s.</td>
</tr>
</tbody>
</table>

*EF₁₃ free drug suspension.

<table>
<thead>
<tr>
<th>Gel formulation</th>
<th>Ciclopirox ethosomal suspension (ml)</th>
<th>Carbopol (%)</th>
<th>Triethanolamine (ml)</th>
<th>Phosphate buffer (pH 7.4)</th>
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<tbody>
<tr>
<td>EG₁</td>
<td>20</td>
<td>1.5</td>
<td>0.5</td>
<td>QS</td>
</tr>
<tr>
<td>*EG-2</td>
<td>0.160g</td>
<td>1.5</td>
<td>0.5</td>
<td>QS</td>
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</table>

*EG-2 free drug gel.
Figure 2: UV Spectrum of Ciclopirox drug.

Figure 3: Calibration curve of Ciclopirox drug in pH7.4 phosphate buffer.

Figure 4: FTIR of Ciclopirox drug.

Figure 5: FTIR of optimized ethosomal gel.
Drug entrapment efficiency: Ciclopirox entrapped within the vesicles was estimated by removing the unentrapped drug. Unentrapped drug was removed by centrifugation of dispersion at 22000rpm at a temperature of 4°C for 45 minutes.

In-vitro evaluation of ethosomes: In-vitro drug release from ethosomes suspension was studied using Franz diffusion cell. The effective area for permeation was 1cm² and diffusion cell receptor volume is 20ml. Egg membrane was used as semi permeable membrane between receptor and donor chambers in Franz diffusion cell. Phosphate buffer (7.4pH) was used as dissolution medium in receptor chamber. Temperature maintained at 37°C and stirring of phosphate buffer in diffusion cell was maintained by using magnetic stirrer at 100rpm. 1-2ml samples were withdrawn through sample port of the Franz diffusion cell at predetermined time point up to 12-24hrs and replace same volume of fresh dissolution medium in diffusion cell.

**Evaluation of ethosomal gel**

**pH of ethosomal gel**: The pH of the gel was measured using a digital pH meter by dipping the glass electrode completely into the ethosomal gel formulation as to cover the electrodes.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Entrapment efficiency (%)</th>
<th>Drug content (%)</th>
<th>pH</th>
<th>Viscosity(cps) at 10 rpm</th>
<th>Spreadability (g/cm/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF1</td>
<td>81.21</td>
<td>98.6</td>
<td>6.6</td>
<td>18124</td>
<td>16.84</td>
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<tr>
<td>EF2</td>
<td>85.63</td>
<td>99.3</td>
<td>6.7</td>
<td>22776</td>
<td>15.92</td>
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<tr>
<td>EF3</td>
<td>87.51</td>
<td>98.6</td>
<td>6.6</td>
<td>20460</td>
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</tr>
<tr>
<td>EF4</td>
<td>89.23</td>
<td>98.3</td>
<td>6.6</td>
<td>13359</td>
<td>15.10</td>
</tr>
<tr>
<td>EF5</td>
<td>85.12</td>
<td>95.3</td>
<td>6.7</td>
<td>12107</td>
<td>19.38</td>
</tr>
<tr>
<td>EF6</td>
<td>92.52</td>
<td>95.6</td>
<td>6.8</td>
<td>18723</td>
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<tr>
<td>EF7</td>
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<td>99.7</td>
<td>7.0</td>
<td>16590</td>
<td>20.30</td>
</tr>
<tr>
<td>EF8</td>
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<td>97.2</td>
<td>6.7</td>
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<td>17.62</td>
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<tr>
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<td>6.8</td>
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<td>17.21</td>
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<tr>
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<td>14321</td>
<td>18.18</td>
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<tr>
<td>EF11</td>
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<td>96.4</td>
<td>6.7</td>
<td>15234</td>
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<tr>
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<td>96.5</td>
<td>6.6</td>
<td>14231</td>
<td>17.25</td>
</tr>
</tbody>
</table>

*Entrapment efficiency is evaluation test for ethosome suspension, remaining evaluation parameters for ethosomal gels.

Table 3: Evaluation test for ethosomes and ethosomal gels.

Figure 6: Scanning electron microscope image for ethosomes.
No. of vesicles (n*)

Average size (d)
Viscosity of the ethosomal gel: Viscosity of ethosomal gel was measured using a Brookfield viscometer, average of 3 measurements was considered as the viscosity of the ethosomal gel.

Drug content: Weighed ethosomal gel was dissolved in methanol and filtered by filter paper to collect filtered drug solution. Filtered drug solution was analyzed using UV visible spectrophotometer at 212nm.

Spreadability: Spreadability was measured using an equipment in which one side was fixed on wooden block and upper side was movable it is tied with weight pan. To calculate spreadability 3-5grams of gel was placed between two slides, weights added to move the slide were noted in below formula.

\[
\text{Spreadability (g.cm/sec)} = \frac{\text{weight tide} \times \text{length moved on glass side}}{\text{time taken to slide}}
\]

Pharmacokinetic studies: In vitro dissolution data of optimized formulation was further investigated by using different release kinetic models.

Results and Discussion

Scanning electron microscope (SEM)

Figure 6

Size distribution analysis

Figure 7

Evaluation of ethosomes and ethosomal gels

Table 3

In-vitro drug release

Figure 8 - 11

Figure 7: Size distribution analysis of formulations EF1-EF12. 

(d_{avg} values for EF1=3.904 µm, EF2=5.32 µm, EF3=5.79 µm, EF4=3.818 µm, EF5=3.72 µm, EF6=3.26 µm, EF7=4.10 µm, EF8=5.45 µm, EF9=5.89 µm, EF10=4.118 µm, EF11=4.12 µm, EF12=3.56 µm).
Pharmacokinetic profile for EF7 ethosomal gel

Table 4

Conclusion

Ciclopirox ethosomes were designed by the method reported by Touitou et al., with few changes. Ethosomes were carried out with ethanol by the sonication at 20%, 30%, 40% and 50% w/w concentrations and Lecithin concentrations varied from 2-4%.

To verify the presence of configuration of vesicular, the designed formulations were observed under microscope at different magnified lenses. The particles size was analyzed.
by specific software for sonicated ethosomes respectively. Vesicular size was found to be in the range of 0 – 5.89 µm. After verification of vesicles and their size, the drug is entrapped by vesicular system and it was evaluated by ultra-centrifugation. Sonicated ethosomes containing 50% ethanol and Lecithin 3% showed higher entrapment value.

In-vitro release was carried out using egg membrane as semi permeable membrane. The optimized gel formulation was found to be zero order. % cumulative drug release into skin was also found to be maximum by the EF7 formulations.

References